

A model for the molecular organization of cytochrome β -561 in chromaffin granule membranes

M. Degli Esposti*, Yu.A. Kamensky, A.M. Arutjunjan and A.A. Konstantinov

*Section of Biochemistry, Dept of Biology, University of Bologna, Bologna, Italy and A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

Received 1 June 1989

The CD spectrum of reduced cytochrome (cyt.) β -561 in chromaffin granule membranes resembles that of mitochondrial cyt. β_1 and indicates possible heme-heme interaction in the protein. Based on spectroscopic data and analysis of the amino acid sequence, a model of cyt. β -561 is suggested, in which the protein carries two transmembrane-localized hemes, each coordinated by two histidines. The model accounts for the presence of two different forms of cyt. β -561 in chromaffin granule membranes and provides a mechanism of transmembrane electron transfer by this hemoprotein.

Cytochrome β -561; Chromaffin granule; Heme-heme interaction; Heme ligand; CD

1. INTRODUCTION

Chromaffin granules from adrenal medulla and some other secretory vesicles specialized in neurotransmitter storage and biosynthesis contain a peculiar hemoprotein called cytochrome b -561 which is believed to function as a transmembrane electron carrier [1].

Hydropathy analysis of the primary structure of cyt. b -561 has led to a model in which a single heme is coordinated to a histidine and methionine residues within the membrane [2].

However, spectroscopic studies favor bis-imidazole heme ligation in cyt. b -561 [3-5]. In addition, the model [2] does not provide an explanation for the presence of two cyt. b -561 species with differing redox [3,6] and spectral characteristics [4] in CGM.

Here, we present a novel model of cyt. b -561

based on protein sequence [2] analysis integrated with results from physico-chemical studies and, in particular, with CD spectra of the cytochrome. CD spectroscopy may provide useful information on the heme environment [7] especially when the protein folding pattern can be deduced from the amino acid sequence (e.g. [8,9]).

2. MATERIALS AND METHODS

CGM were isolated from bovine adrenal medulla according to [10]. Beef heart complex cyt. bc_1 [11] was assayed spectrally as described [8,9].

CD spectra were recorded in a Jobin Yvon mark III dichrograph (in Moscow) or in a Jasco J-500 spectropolarimeter with computer-aided data collection (in Bologna).

The dipole strength and rotational strength of the absorption and CD bands in the Soret region were calculated in cgs units [12]. Exciton coupling calculations were carried out utilizing the point monopole approximation and assuming negligible cancellation of the four possible couplings of the degenerated transitions of two identical hemes [13]. The di-heme system was considered to possess a C₂ symmetry axis along the membrane normal in order to simplify calculations [12-14] in agreement with the current models of mitochondrial-type di-heme cytochromes b [15,16].

The hydropathy profile of cyt. b -561 [2] was evaluated using statistical methods [17,18]. The possible locations of the heme-binding sites were investigated using two procedures specified in the legend to table 2.

Correspondence address: A.A. Konstantinov, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 119899, USSR

Abbreviations: CD, circular dichroism; MCD, magnetic circular dichroism; CGM, chromaffin granule membranes; cyt, cytochrome

3. RESULTS AND DISCUSSION

3.1. Heme-heme interaction in cyt. *b*-561

Redox titrations reveal two cyt. *b*-561 components in CGM with different E_m values [3,6] and spectral characteristics [4]. This may indicate cyt. *b*-561 as being a two-heme protein similarly to the mitochondrial-type cyt. *b*. In the latter case heme-heme interaction could be elicited by CD spectroscopy [8,9].

Fig.1 shows CD spectra of CGM cyt. *b*-561. Notably, whereas the dipole strength of the cyt. *b*-561 Soret band decreases by $\sim 1/3$ upon reduction (cf. [19,20]), the CD spectral intensity increases, which is unusual for a single-heme protein (e.g. table 1). Peculiarly, the CD spectrum of reduced cyt. *b*-561 resembles that of the mitochondrial cyt. *b* low-potential heme (b_1) (table 1) known to contain a significant contribution from excitonic coupling with the second *b* heme (*b*-562) [8]. Accordingly, the large increase in the main positive band in the CD spectrum of cyt. *b*-561 upon reduction may also point to a substantial excitonic coupling signal added to the individual CD bands of the high- and low-potential cyt. *b*-561 hemes.

Semiquantitative calculations assuming two interacting hemes and considering geometrical situations giving rise to exciton coupling bands stronger than the positive CD signal of the reduced cyt. *b*-561 (1.6×10^{-39} cgs, fig.1B) indicate a minimal Fe-to-Fe distance of 21 Å provided the tilt between the hemes is $> 10^\circ$, or up to 32 Å for larger values of the tilt angle.

3.2. Axial ligands and potential heme-binding sites in cyt. *b*-561

The absorption spectra of oxidized cyt. *b*-561 do not show any signs of an approx. 700 nm band (not shown; [5]) (cf. [19]) which rules out the possibility of methionine being the 6th axial heme ligand as suggested in [2]. On the other hand, very close similarity of the reduced cyt. *b*-561 MCD spectrum β -band with that of cyt. *b*₅ or the bis-imidazole complex of protoheme [3-5,22] makes lysine ligation unlikely (Lys and His axial ligands give a markedly different fine structure of the magneto-optical spectral β -band [22]). Accordingly, His imidazoles are the most likely axial ligands for cyt. *b*-561 heme iron.

We found several His-containing peptides in cyt.

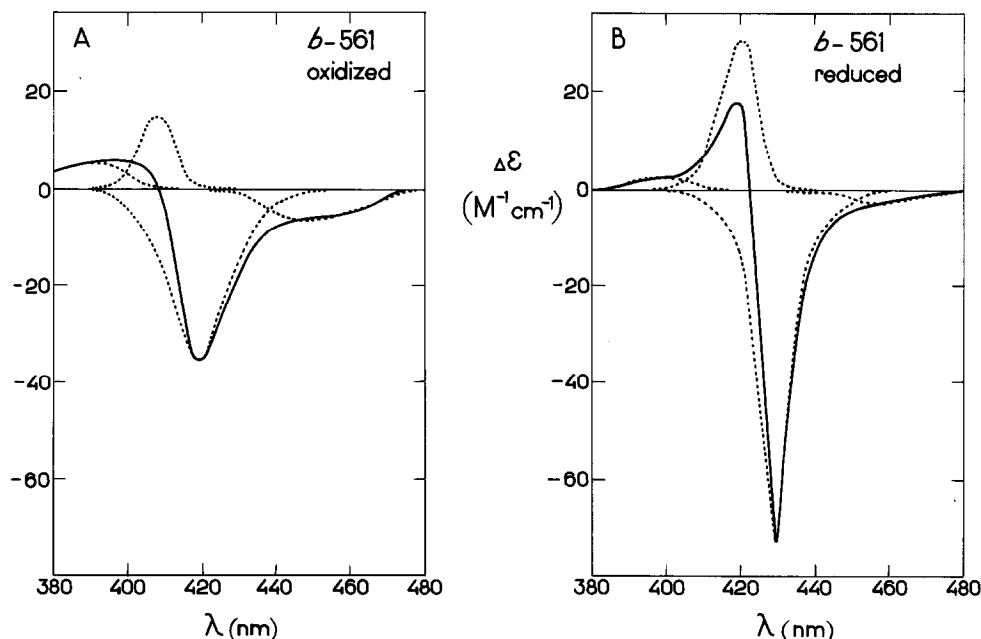


Fig.1. CD spectra of CGM cytochrome *b*-561 in the Soret region. Conditions: CGM, 1.3 mg protein/ml ($\sim 5 \mu\text{M}$ *b*-561) in 0.3 M sucrose, 10 mM Mops (pH 7.2), 0.1 mM EDTA. (A) Oxidized sample (+ 40 μM Co(phenanthroline)₃Cl₃); (B) dithionite-reduced sample. Dotted lines are the Gaussians into which the experimental signals were tentatively deconvoluted.

Table 1
Comparison of the CD properties of cytochrome *b*-561 from chromaffin granules with those of other *b*-type cytochrome

Cytochrome	Redox state	Dipole ^a strength	CD features				
			Positive band ^b		Crossover (nm)	Negative band	
			λ_{\max} (nm)	R^c		λ_{\max} (nm)	R^c
Cyt. <i>b</i> -561, chromaffin granules	ox	10.52	409	1.01	408	420	-3.93
	red	7.17	421	2.68	426	430	-4.09
Cyt. <i>b</i> ₁ , beef heart <i>bc</i> ₁ complex	ox	9.48	413	2.77	415	425	-10.35
	red	8.14	421	2.90	426	431	-4.79
Cyt. <i>b</i> -562, <i>E. coli</i> ^e	ox	7.65	398	0.59	403	421	-2.08
	red	7.09	negligible		399	423	-0.74
Cyt. <i>b</i> -555 (<i>b</i> ₅), house fly larvae ^f	ox	7.37	negligible		402	416	-1.98
	red	6.61	negligible		418	427	-1.4

^a Intensity of the optical Soret band ($\times 10^{-35}$, in cgs units) calculated as in [12]

^b Main positive and negative band deconvoluted from the experimental CD signals in the Soret region (cf. fig. 1)

^c Rotational strength of the deconvoluted CD band ($\times 10^{-39}$, in cgs units), calculated by Gaussian approximation as in [12]

^d Optical properties of *b*₁ have been considered as the mean of those of the two *b*-hemes in the *bc*₁ complex. For the CD properties, see [9]

^e Data calculated from the spectra in [19] for this soluble monoheme cytochrome

^f Data calculated from the spectra in [20]. The CD spectra of calf liver cyt. *b*₅ are less intense than those of cyt. *b*-555 [21]

b-561 that exhibit substantial homology with the presumed His-ligand peptides of mitochondrial and other related membrane cytochromes *b* (table 2). Of the seven histidines in the protein, H109, H113 and H143 have the high probability of being the heme ligands (table 2), H109 and H113 being too close to each other for coordination to the same heme. H130 and H131 show very low 'histidine index' and homology and may be excluded, but H75 and H182 can also be considered as potential heme ligands (table 2).

A hydropathy profile analysis of the amino acid sequence according to [17,18] suggests 6 transmembrane helices in cyt. *b*-561 in agreement with [2] with both N- and C-termini at the cytoplasmic side. According to this folding model, H109, H113 and H182 are located near the cytoplasmic (negative) and H75 and H143 near the luminal (positive) membrane surfaces.

A hypothetical structure for cyt. *b*-561 as a two-heme protein based on the above considerations and on analogy with other energy-transducing cytochromes *b* [15,16] is shown in fig.2.

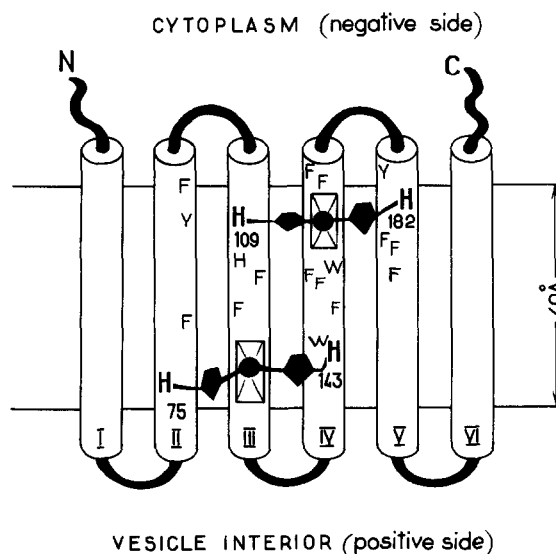


Fig.2. Model for the molecular organization of cyt. *b*-561 of chromaffin granules. The proposed histidine ligands of the heme irons and aromatic residues close to the hemes are indicated using the single-letter code. The approximate dimensions of the hemes and the membrane are respected in the model.

Table 2
Screening of the possible ligands of the heme(s) in cyt. *b*-561 of chromaffin granules

Peptides			Histidine index ^a	SER ^b	Similarity ^c score	Similar ligand peptides ^d , iron center and species	
-4	0	+4					
QFNV	H75	PLCM	0.56	2	7	non-haem	<i>R.sphaeroides</i> , <i>R.cap-sulatus</i> and <i>Rhodo-pseudomonas viridis</i>
TKVL	H109	GLLH	1.04	4	9	<i>b</i> ₁ , <i>bc</i> ₁	<i>T.brucei</i> and <i>L.taran-tolae</i>
HGLL	H113	VFAF	0.99	2	9 8	<i>b</i> _h , <i>bc</i> ₁ <i>b</i> _h	<i>A.nudilans</i> ; 3 higher plants, <i>S.pombe</i> and <i>N.crassa</i>
AVFE	H130	HRKK	0.30	0	—	none	
VFEH	H131	RKKG	0.25	3	—	none	
LYSL	H143	SWCG	0.83	5	7	<i>b</i> ₆ , <i>b</i> ₆ ^f <i>b</i> ₁ , <i>bc</i> ₁	Spinach and tobacco; maize and <i>O.villancae</i>
YRPQ	H182	VFFG	0.58	2	8	<i>b</i> ₁ , <i>bc</i> ₁ <i>b</i> ₁ , <i>bc</i> ₁	<i>L.tarantolae</i> ; <i>T.brucei</i>

^a Sum of the frequency of occurrence of residues in positions -3 to +3 calculated in a database formed by 90 proposed ligand peptides of cyt. *b*, *b*₆ and *b*-559 [23]

^b Number of specifically enriched residues (SER), i.e. residues that are more concentrated in one position of the ligand peptides of cyt. *b*, *b*₆, *b*-559 and the non-heme irons in bacterial reaction centers than their expected transmembrane random distribution. When this parameter is larger than or equal to 4, there is over 80% probability for being a heme-ligand peptide (Degli Esposti, M., unpublished)

^c Similarity measured in the aligned nonapeptides with no gap using the method in [24]. Significant sequence homology is seen when the score is larger than 6 [24]

^d Peptides proposed or known to bind membrane-buried iron centers that show a score of similarity higher than 6. The center and the species are indicated

Notably, the two hemes localized at the opposite sides of the membrane have rather different surroundings. A large number of aromatic residues around the negative side-exposed heme may entail its dominating contribution to the CD spectra of CGM cyt. *b*-561.

The advantages of the model in fig.2 as compared to that in [4] are as follows:

- Bis-imidazole heme ligation is consistent with spectroscopic data available, whereas the His, Met coordination suggested in [2] can be ruled out [5].
- Rather different protein environment of the two hemes (fig.2) can explain the presence of CGM of two cyt. *b*-561 species with differing redox [3,6] and spectral [4] characteristics.
- The two-heme structure, analogous to that of mitochondrial-type cyt. *b* [15,16], provides the basis for a mechanism of transmembrane electron transfer via cyt. *b*-561 [1]. The Fe-to-

Fe distance between the hemes of ~30 Å predicted by the model agrees with that deduced from heme-heme interaction revealed by CD spectra.

- Location of at least one *b*-561 heme very near to the cytoplasmic (negative) surface of CGM is corroborated by an extremely high reactivity of the cytochrome with membrane-impermeable electron donors and acceptors [3].

Acknowledgements: We are grateful to Dr M. Crimi for computing assistance and to Professor V.P. Skulachev for his interest in this work and critically reading the manuscript.

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